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Analysis of PCBs in Waters

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26.1 Introduction

PCB stands for polychlorinated biphenyls or simply chlorinated biphenyls. Though PCBs can be considered as part of a broader group of chemicals known as chlorinated hydrocarbons, considering the sources, the fate, and the behavior of PCBs in the environment, they have been included among the persistent organic pollutants (POPs). The main features of these pollutants are that they are toxic, persistent, bioaccumulative, and prone to long-range transport.

PCBs were intensively produced worldwide between the 1930s and the 1980s owing to their remarkable chemical and physical features, including a high dielectric constant, a high solubility in hydrocarbons and almost insolubility in water, a very low volatility, and a high chemical stability and heat resistance (Shiu and Ma 2000; Li et al. 2003). Their uses include dielectric fluids in electric equipment (transformers and capacitors), adhesives, plastics, or thermal insulation materials. According to the literature (de Voogt and Brinkman, 1989), the cumulative global production was estimated to be on the order of 1.5 million tons in the 1980s, and half of it is attributed to Monsanto (USA).

The presence of PCBs in remote regions including the Arctic and Antarctic is a consequence of their large-range transport potential (Schringer 2009) and persistence, though the use and marketing have been
heavily restricted in Europe*,† and USA‡ since the early 1980s. This fact, together with the awareness that arose by the presence of these POPs in the food chain and the effects on the environmental (Eisler 1986) and human health (US EPA, 1996), have served to demand environmental monitoring programs (Stockholm Convention§ or Arctic Monitoring and Assessment Program, AMAP (De Wit and Muir 2010)) and the elaboration of environmental inventories (Hornbuckle et al. 2006; Breivik et al. 2007).

Though chemically there are 209 congeners (see Figure 26.1), the production of PCBs consisted of the progressive chlorination of biphenyl up to the desired content on chlorine, and therefore they were manufactured as commercial mixtures with a different distribution of a limited amount of congeners. As a result, the number of congeners found in the environmental compartments is much lower than those 209. In this sense, based on the toxic mode of action, PCBs are frequently distinguished between the nonortho or mono-ortho PCBs, with a toxicity close to that of dioxins, and the rest. Therefore, the former group of PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) are included in many monitoring programs, and from the other PCBs, only a few (28, 52, 101, 118, 138, 153, and 180) are selected to cover a wide range of chlorination degrees (UNEP 2007; van Leeuwen and de Boer 2008).

A great amount of analytical methods are reported in the literature, including both the standard operating procedures generally applied in surveillance and monitoring procedures (i.e., US EPA, ISO and Ospar or Helcom commissions methods), and the specific research developments (Fuoco and Ceccarini. 2000; Boer and Law 2003; De Zaater et al. 2005; Hornbuckle et al. 2006; Muir and Sverko 2006; Reiner et al. 2006). However, the implementation of routine analytical methods is still limited to a few laboratories, especially if we consider not only the low concentrations that these compounds are expected to be found in the water courses or the sea water column (from femtograms to nanograms per liter, as reported in the Great Lakes (USA) (Hornbuckle et al. 2006)), but also in particular the behavior of many POPs in water–sediment–atmosphere interfaces and the role that the solid particles and the organic matter play (Lohman et al. 2007). In addition to this, the requirements of global monitoring plans include the use of harmonized protocols to identify trends in levels over time and to provide information about the regional and global transport (UNEP 2007), which means several analytical challenges.

One of the key aspects to understand the technical requirements and the analytical outputs is the framework under which the analysis of PCBs is accomplished. Though in the literature a large variety of methods can be found (Erikson 1997), and the analytical background is consolidated in many labs, the specificity of each regulation, especially for the official laboratories taking part in surveillance and international monitoring, makes the comparison unrealistic. In addition to this, one of the best approaches to fulfill the requirements to analyze the PCBs mentioned above (both the 7 ortho and the 12 nonortho, and polychlorinated dibenzodioxins/polychlorinated dibenzo-furans (PCDD/PCDF) as well) makes use

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§ The Stockholm convention on persistent organic pollutants (POPs), 2001 (www.pops.int).
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of labeled isotopes and high-resolution mass spectrometry (HRMS), which is only available in high-standard labs (UNEP 2007). For less demanding analysis, standard chromatographic techniques, such as gas chromatography–mass spectrometry (GC–MS) or even gas chromatography–electron capture detector (GC–ECD), can fulfill the analysis of most PCBs.

Considering the framework proposed in the Guidance on the Global Monitoring Plan for Persistent Organic Pollutants (UNEP 2007) as a reference, the analytical procedure includes the sampling, extraction, and separation/quantification steps. In the following sections, the main procedures that have been proposed lately (2005–2011) will be described.

### 26.2 Sampling

Though it is an all-known key step in every analysis, it is usually covered with a lower level in many reviews and procedures compared to the coverage of sample treatment or instrumental analysis. Broadly speaking, we can distinguish two meanings under this section. One is directly related to the sampling plan, that is, the statistical considerations that are behind highly cost–labor issues such as the frequency of the sampling, or the size of samples that is taken. The other is an operatively driven matter, which considers how samples are taken.

Since the statistical background of sampling is out of the scope of this chapter, we can only address further readings on these matters (Popek 2003; Zhang 2007). Regarding the technical details of the sampling issues, up to the breakthrough of the passive sampling techniques (Huckins et al. 2006), only “active” techniques were discussed.

When active samplings are required, Niskin or Go-flo bottles are typically used and the main considerations are the cleanliness and the ease of use. In the case of PCBs, as many other hydrophobic POPs, plastic materials should be avoided for sampling and sample storage due to the adsorption of target analytes onto the container materials (Lysiak-Pastuszak and Kryseel 2004). In this sense, Teflon-lined materials are highly recommended. In addition to this, water samples should be extracted as soon as possible and be used with as little manipulation as possible. The use of thorough cleaning procedures and procedural blank values allows the achievement of the low detection limits required in these analyses.

Before dealing with a detailed description of the chemical procedures used in the determination of PCBs in aquatic media, it is better to describe the main contributions of the passive sampling techniques. These techniques are based on the free diffusion of analytes from the aquatic medium to the sampling device (usually a solid phase) as a result of a difference between the chemical potentials of the analyte in the two media (Vrana et al. 2005). They have been thoroughly described in the literature (Huckins et al. 2006; Greenwood et al. 2007). In the recent literature, the field performance of several sampling devices (semipermeable membrane device (SPMD), membrane enclosed sorptive coating samplers (MESCO), and Chemcatcher for monitoring hydrophobic contaminants (Allan et al. 2009) have been compared. These provide data that are less variable than for the active sampling methods, especially because the presence of suspended particulate matter affects the latter more strongly. SPMD have been used in the determination of polycyclic aromatic hydrocarbons (PAHs), PCBs, and organochlorine pesticides in river water in China (Wang et al. 2009a) with an exposure time of 24 days. In this work, the use of performance reference compounds (PRC) is discussed (Huckins et al. 2002) to compensate the effects of hydrodynamic or temperature variations and those of biofouling, which can be very severe. A similar approach was used in the monitoring of surface waters in the Biosphere Reserve of Krivoklatsko (Czech Republic) (Koci et al. 2007), but in this particular work, the interpretation of the results in terms of toxicity showed the suitability of passive samplers to rank the toxicity of contaminated sites when no living organisms can be exposed.

### 26.3 Extraction

The determination of PCBs in surface water requires an extraction step prior to chromatographic/electrophoretic separation and detection. The extraction step is intended to either preconcentrate the analytes
or eliminate or simplify the sample matrix, getting rid of some of the matrix components (Mitra 2003). Some other minor applications include the adaptation of the solvent to a given instrumental method or the purification of the analyte for further characterizations.

Classical extraction techniques include liquid–liquid extraction (LLE) and solid-phase extraction (SPE).

### 26.3.1 Liquid–Liquid Extraction

LLE is still often used for the determination of PCBs as can be seen in the applications included in Table 26.1.

Typical water volumes used during LLE of PCBs range from 200 mL to 1 L (García-Flor et al. 2005; Zaater et al. 2005; Rissato et al. 2006; Ozcan et al. 2009; Dasgupta et al. 2011; Ozcan, 2011), although up to 10 L have also been applied by Moret and coworkers (Moret et al. 2005) in the determination of PCBs in surface water from the Venice lagoon.

For elution, dichloromethane has been mostly used (García-Flor et al. 2005; Rissato et al. 2006; Ozcan et al. 2009; Dasgupta et al. 2011; Ozcan, 2011), although it has also been used combined with other solvents as in the mixtures of dichloromethane:pentane (6:4, v/v) (Zaater et al. 2005) and dichloromethane (2:1) (Moret et al. 2005). In general, extraction of the sample was performed in triplicate with volumes in the 20–75 mL range (García-Flor et al. 2005; Rissato et al. 2006; Ozcan et al. 2009; Dasgupta et al. 2011; Ozcan 2011) in each of the consecutive extractions.

In a few cases, a clean-up or fractionation step was performed after the LLE preconcentration step. García-Flor and coworkers (García-Flor et al. 2005) used 3 g of neutral alumina, deactivated with 3% water (w/w) and the PCBs were eluted with 10 mL of n-hexane. Similarly, Moret et al. (2005) used a column filled with 2 cm of Florisil and 1 cm of alumina for the clean-up of the LLE extract of surface waters of the Venice lagoon and PCBs were eluted with 30 mL n-hexane.

### 26.3.2 Solid-Phase Extraction

Nowadays, SPE is surely the most widely used technique, essentially due to its strengths in selectivity, flexibility, and high automation potential.

SPE involves the use of a chromatographic sorbent in a column format or filter. A sample is passed through the cartridge or filter, the analytes are retained on the sorbent and the liquid matrix passes through. Then, if required, the sorbent bed is washed to remove undesired interferences, and the purified analytes are subsequently eluted from the column.

For the determination of PCB in water, reverse-phase SPE (RP-SPE) is used since this is the way to assure the highest recoveries due to the higher affinity of the analytes toward the solid phase than the aqueous media. Examples of common sorbents used in nonpolar SPE include C18, C8, C6, C4, C2, phenyl, cyclohexyl, and proplycyano. These nonpolar groups are chemically attached on the surface of a silica particle. In addition to this, many commercial nonpolar polymers have been used for the same purposes. In the case of PCBs, C18 SPE has been often used (Almeida et al., 2007; Pitarch et al. 2007; Ozcan et al. 2009; Prieto et al. 2010; Dasgupta et al. 2011; 2010; Portoles et al. 2011), mostly in 200 mg (Prieto et al. 2010) and 500 mg (Almeida et al. 2007; Pitarch et al. 2007, 2010; Portoles et al. 2011) cartridge format. In the case of polymeric phases, Sánchez-Avila et al. used a 200 mg Oasis HLB cartridge for the analysis of PCBs together with other organic micropollutants.

The loading water volumes ranged between 100 and 500 mL (Prieto et al. 2010), though higher volumes, up to 1000 mL, have also been used (Sánchez-Avila et al. 2011). To release the PCBs from the solid phases, nonpolar solvents are typically used. In the case of C-18 cartridges, elution solvents or mixtures such as n-hexane:ethyl acetate (7:3, v/v) (Ozcan et al. 2009; Prieto et al. 2010; Dasgupta et al. 2011), ethyl acetate:chloroform (1:1, v/v) (Pitarch et al., 2007, 2010; Portoles et al., 2011), or ethyl acetate (Almeida et al., 2007) have been used. In the case of Oasis HLB (Sánchez-Avila et al. 2011), n-hexane:chloroform (1:1, v/v) followed by chloroform:acetone (1:1, v/v) were used.

Although most of the applications revised recently have included the use of cartridges, Zorita and coworkers (Zorita et al. 2006) used C18 filters. The SPE step was followed by supercritical fluid
### TABLE 26.1
Summary of the Most Common Extraction and Analysis Methodologies Used in the Determination of PCBs in Water and Their Respective Limits of Detection (LOD), Recoveries, and Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sampling Technique</th>
<th>Preconcentration Technique</th>
<th>Clean-Up</th>
<th>Analysis</th>
<th>LOD</th>
<th>Recovery</th>
<th>Precision</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea surface microlayer</td>
<td>LLE</td>
<td>1 L with 60 mL DCM ×3</td>
<td>Alumina</td>
<td>GC–ECD</td>
<td>0.001–0.05 ng/L (LOQ)</td>
<td>88</td>
<td>15</td>
<td>García-Flor et al. (2005)</td>
</tr>
<tr>
<td>Surface water</td>
<td>SPMD</td>
<td>Dialysis</td>
<td>Silica gel deactivated with H₂SO₄</td>
<td>GC–MS/MS</td>
<td></td>
<td></td>
<td></td>
<td>Koci et al. (2007)</td>
</tr>
<tr>
<td>Surface water (lagoon)</td>
<td>LLE</td>
<td>10 L with 200 mL pentane:DCM (2:1)</td>
<td>Florisil + alumina</td>
<td>GC–MS</td>
<td>6–8 pg/L</td>
<td>88.6–105.8</td>
<td>6.2–10.2</td>
<td>Moret et al. (2005)</td>
</tr>
<tr>
<td>Estuarine water</td>
<td>SPME</td>
<td>35°C, 1000 rpm, 30 min</td>
<td></td>
<td>GC–ECD</td>
<td></td>
<td></td>
<td></td>
<td>Nie et al. (2005)</td>
</tr>
<tr>
<td>Tap water, well water, domestic and industrial wastewater</td>
<td>USAEME</td>
<td></td>
<td></td>
<td>GC–MS</td>
<td>14–30 ng/L</td>
<td>80–100</td>
<td>&gt;10</td>
<td>Ozcan et al. (2009)</td>
</tr>
<tr>
<td>Tap water, well water, domestic and industrial wastewater</td>
<td>SPE  C₁₈</td>
<td></td>
<td></td>
<td>GC–MS</td>
<td></td>
<td></td>
<td></td>
<td>Ozcan et al. (2009)</td>
</tr>
<tr>
<td>Tap water, well water, domestic and industrial wastewater</td>
<td>LLE 200 mL with 20 mL DCM ×3</td>
<td>GC–MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ozcan (2011)</td>
</tr>
<tr>
<td>Surface, ground, wastewater</td>
<td>SPE  C₁₈, 200 mL</td>
<td></td>
<td></td>
<td>GC–MS/MS</td>
<td>5–150 ng/L</td>
<td>70–182</td>
<td>5–19</td>
<td>Pitarč et al. (2007)</td>
</tr>
<tr>
<td>Surface water (river)</td>
<td>LLE</td>
<td>1 L with 75 mL DCM ×3</td>
<td></td>
<td>GC–MS</td>
<td>2–3 ng/L</td>
<td>77–119</td>
<td>5.9–12.5</td>
<td>Rissato et al. (2006)</td>
</tr>
<tr>
<td>Surface water (river)</td>
<td>HS-SPME</td>
<td>PANI</td>
<td></td>
<td>GC–μECD</td>
<td>0.05–0.1 ng/L</td>
<td>83.0–110.7</td>
<td>5.3–9.4</td>
<td>Wang et al. (2009b)</td>
</tr>
<tr>
<td>Surface water</td>
<td>LLE</td>
<td>500 mL with 40 mL, 30 mL, 30 mL, DCM:petroleum ether (60:40, v/v)</td>
<td></td>
<td>GC–MS</td>
<td>80–200 ng/L (instrumental??)</td>
<td>84.6–91.9</td>
<td>3.5–9.4</td>
<td>Zaater et al. (2005)</td>
</tr>
<tr>
<td>Estuarine water</td>
<td>SPE  C₁₈</td>
<td>LVI–GC–MS</td>
<td></td>
<td>GC–MS</td>
<td>0.04–0.21 ng/L</td>
<td>88.5–113.9</td>
<td>1.3–9.0</td>
<td>Almeida et al. (2007)</td>
</tr>
<tr>
<td>Coastal seawater</td>
<td>Porous polypropylene hollow fiber membrane coated with a conjugated polymer hexane</td>
<td>GC–MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basheer et al. (2007)</td>
</tr>
</tbody>
</table>
### TABLE 26.1 (continued)

Summary of the Most Common Extraction and Analysis Methodologies Used in the Determination of PCBs in Water and Their Respective Limits of Detection (LOD), Recoveries, and Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sampling Technique</th>
<th>Preconcentration Technique</th>
<th>Clean-Up</th>
<th>Analysis</th>
<th>LOD</th>
<th>Recovery</th>
<th>Precision</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal seawater</td>
<td>SPME</td>
<td>PDMS, 1–1.1 mm, 30 min, no pH salt conditioning</td>
<td>GC–MS</td>
<td>0.14–6.74 ng/L</td>
<td>91.2–105.6</td>
<td>4.1–10.1</td>
<td>Basheer and Lee (2004)</td>
<td></td>
</tr>
<tr>
<td>Tap and lake water</td>
<td>DLLME</td>
<td>0.1 g NaCl, 8 μL 1-undecanol, 1 mL ACN, 3500 rpm, 2 min</td>
<td>GC–ECD</td>
<td>3.3–5.4 ng/L</td>
<td>79.4–112.2</td>
<td>5.8–8.8</td>
<td>Dai et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>LLME</td>
<td>250 μL chloroform, 6000 rpm, 5 min</td>
<td>GC–ToF-MS</td>
<td>76–120</td>
<td>10</td>
<td>Dasgupta et al. (2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>LLE</td>
<td>200 mL with 20 mL DCM ×3 USEPA3510C</td>
<td>GC–ToF-MS</td>
<td>76–120</td>
<td>10</td>
<td>Dasgusta et al. (2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>SPE</td>
<td>C18, 200 mL</td>
<td>GC–ToF-MS</td>
<td>76–120</td>
<td>10</td>
<td>Dasgusta et al. (2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuarine water and seawater</td>
<td>MASE</td>
<td>High-density polypropylene, 4 cm long with a wall thickness of 0.03 mm and an internal diameter of 6 mm, 50:50 ethyl acetate:cyclohexane 800 μL, 50°C, 60 min, 500 rpm</td>
<td>LVI–PTV–GC–MS</td>
<td>0.6–31.6 ng/L</td>
<td>81–120</td>
<td>3–19</td>
<td>Prieto et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Influent, effluent, snow</td>
<td>MEPS</td>
<td>2 mg, C18</td>
<td>LVI–GC–MS</td>
<td>0.2–26.1 ng/L</td>
<td>78–91</td>
<td>&lt;21</td>
<td>Prieto et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Influent, effluent, snow</td>
<td>SPE</td>
<td>C18, 200 mg</td>
<td>LVI–GC–MS</td>
<td>0.2–26.1 ng/L</td>
<td>78–91</td>
<td>&lt;21</td>
<td>Prieto et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>River water</td>
<td>HS-SPME</td>
<td>PDMS 100 μm, 80°C, 60 min, no salt</td>
<td>GC–MS</td>
<td>0.9–16.2</td>
<td>75.3–99.3</td>
<td>5.34–18.37</td>
<td>Derouiche et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>Coastal water</td>
<td>PUF</td>
<td>PUF: Soxhlet, toluene</td>
<td>Silica</td>
<td>98</td>
<td>85.9–92.0</td>
<td>3.4–5.8</td>
<td>Josefsson et al. (2011)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POM</td>
<td>POM, shaking, n-hexane</td>
<td>Activated carbon</td>
<td>98</td>
<td>85.9–92.0</td>
<td>3.4–5.8</td>
<td>Josefsson et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>River water</td>
<td>Dynamic HF-LPME</td>
<td>toluene, 3 μL, 15 min, dwell time 8 s, 45°C, no salt, 500 rpm</td>
<td>GC–MS</td>
<td>13–41 ng/L</td>
<td>85.9–92.0</td>
<td>3.4–5.8</td>
<td>Li et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Type of Water</td>
<td>Method</td>
<td>Volume</td>
<td>Extraction Solvent</td>
<td>Other Conditions</td>
<td>Concentration (ng/L)</td>
<td>Recovery (%)</td>
<td>LOQ (ng/L)</td>
<td>Authors</td>
</tr>
<tr>
<td>---------------------------------------</td>
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</tr>
<tr>
<td>Sediment pore water</td>
<td>SPME VALLME</td>
<td>5 mL, 200 μL chloroform, vortex 2 min 3000 rpm, centrifuge 4000 rpm, 5 min, no salt</td>
<td></td>
<td>GC–MS</td>
<td>0.36–0.73</td>
<td>96</td>
<td>5</td>
<td>Maruya et al. (2009)</td>
</tr>
<tr>
<td>Tap, well, surface, bottled waters, and municipal, treated municipal, and industrial wastewaters</td>
<td>LLE US EPA Method 3510C</td>
<td>200 mL, 30 mL DCM ×3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ozcan (2011)</td>
<td></td>
</tr>
<tr>
<td>Seawater and interstitial marine water</td>
<td>SBSE</td>
<td>10 mL, PDMS 20 mm² × 10 mm, 100 g/L NaCl, 14 h</td>
<td></td>
<td>TD–GC–MS</td>
<td>0.4–2.7</td>
<td>56.3–83.4</td>
<td>1.5–6.0</td>
<td>Pérez-Carrera et al. (2007)</td>
</tr>
<tr>
<td>Wastewater</td>
<td>SPE</td>
<td>500 mg C18</td>
<td></td>
<td>GC–MS/MS</td>
<td></td>
<td></td>
<td></td>
<td>Pitarch et al. (2010)</td>
</tr>
<tr>
<td>River water and groundwater</td>
<td>SBSE</td>
<td>PDMS 10 × 0.5 mm, 2 h, 8 mL + 2 mL MeOH, 1000 rpm</td>
<td></td>
<td>TD–GC–MS</td>
<td>0.05–0.15</td>
<td>28–93</td>
<td>3.3–29.7</td>
<td>Popp et al. (2005)</td>
</tr>
<tr>
<td>Surface water, groundwater, effluent, wastewater</td>
<td>SPE</td>
<td>500 mg, C18, 250 mL</td>
<td></td>
<td>GC–TOF/MS</td>
<td>21–1000 ng/L (LOI)</td>
<td></td>
<td></td>
<td>Portoles et al. (2011)</td>
</tr>
<tr>
<td>River, seawater</td>
<td>DLLME Acetone disperser 500 μL chlorobenzene 10 μL extraction solvent, 2 min centrifuge 5000 rpm, no salt</td>
<td></td>
<td></td>
<td>GC–ECD</td>
<td>&lt;2</td>
<td>92–114</td>
<td>4.1–11</td>
<td>Rezaei et al. (2008)</td>
</tr>
<tr>
<td>Seawater, river water, wastewater treatment plant (WWTP) effluents</td>
<td>SPE</td>
<td>200 mg Oasis HLB, 100–250 mL</td>
<td></td>
<td>GC–MS/MS</td>
<td>0.2–27 ng/L (MDL)</td>
<td>73–116</td>
<td>1–19</td>
<td>Sánchez-Avila et al. (2011)</td>
</tr>
<tr>
<td>Coastal water</td>
<td>SBSE 20 × 10 mm, PDMS, no NaCl, 10% MeOH, 24 h, 900 rpm</td>
<td></td>
<td></td>
<td>TD–GC–MS</td>
<td>0.011–0.194</td>
<td>86–118</td>
<td>2–24</td>
<td>Sánchez-Avila et al. (2011)</td>
</tr>
<tr>
<td>Wastewater</td>
<td>SPE–SFE filter + SFE</td>
<td>100°C and 35 MPa for 45 min at a flow rate of 1.5 mL/min. Collection was done in 10 mL 2-methyl heptane at a temperature of −5°C</td>
<td></td>
<td>Dual column GC–ECD</td>
<td>101–112</td>
<td>1–5</td>
<td>Zorita et al. (2006)</td>
<td></td>
</tr>
</tbody>
</table>

extraction (SFE) with CO₂ at 100°C, 35 MPa for 45 min at 1.5 mL/min. Collection of the SFE extract was performed in 10 mL of 2-methyl-heptane at −5°C.

26.4 Novel Extraction Techniques

In the last few decades, different extraction techniques for the analysis of organic pollutants, including PCBs in water, are being developed to improve the recoveries and the sensibility and to ease the lab work reducing solvent consumption and using even solventless procedures. The extraction techniques that try to minimize solvent consumption can be classified into two main groups: (i) sorptive extractions and (ii) liquid–liquid microextraction. Among sorptive extractions, solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE) and microextraction by packed sorbent (MEPS) are included. Membrane liquid-phase microextraction (MLPME) and dispersive liquid–liquid microextraction (DLLME) are classified in the group of liquid–liquid microextraction techniques (LLME) (Etxebarria et al., 2012).

26.4.1 SPME and SBSE

In SPME and SBSE, the analytes are extracted from the aqueous matrix into a nonmiscible liquid or solid polymer, mainly liquid polydimethylsiloxane (PDMS). Their principles and applications have been reviewed elsewhere (Baltussen et al. 2002; Prieto et al. 2010). The major difference between SPME and SBSE relies on the amount of polymeric phase. While the volume of the fiber is usually 0.5 μL in SPME, the smallest stir-bar has roughly 24 μL. Among other variables, extraction efficiency is dependent on the amount of polymeric acceptor phase and, thus, while SPME is a microextraction technique and repeated extraction can be performed on the same sample, the recoveries are much higher in SBSE.

Working with either SPME or SBSE requires the two typical steps of any separation technique: extraction and desorption. In this particular case, it makes sense to perform the extraction under kinetic conditions and not only under equilibrium. Additionally, the polymeric phase can be directly immersed in the water sample (direct SPME or SBSE) or located in the headspace (HS-SPME or HSSE) for more volatile compounds. In the case of SPME, the headspace (HS-SPME) (Derouiche et al. 2007; Wang et al. 2009b) and immersion mode (SPME) (Nie et al. 2005; Basheer et al. 2007) are described in the literature, whereas in the case of SBSE, all the applications found in the literature for PCBs in water used the immersed mode (Popp et al. 2005; Pérez-Carrera et al. 2007, 2008; Sánchez-Avila et al. 2010, 2011). In both cases, the required volumes of aqueous solutions ranged from 2 to 100 mL, according to the type of extracting device (SPME or SBSE) and the analytical requirements.

From the different polymeric phases commercially available in SPME, PDMS/divinylbenzene (DVB), polyacrylate (PA), carboxen/PDMS, carbowax (CW)/DVB, CW/template resin, and DVB/carboxen/PDMS, PDMS has been most widely used (Basheer et al. 2007; Derouiche et al. 2007), although polyaniline (PANI)-based polymeric phases have also been recently used (Wang et al., 2009b). Basheer and coworkers (Basheer et al. 2007) studied PDMS, PDMS-DVB, PA, and PDMS provided the best extraction yields. Similarly, Derouiche and coworkers (Derouiche et al. 2007) also studied fibers of different thicknesses (100, 30, and 7 μm PDMS), and 65 μm PDMS-DVB and 100 μm PDMS provided the best results. An increase of extraction efficiency was observed with an increase in the thickness of the polymeric phase. In the case of SBSE, PDMS is also mostly used (Popp et al. 2005; Pérez-Carrera et al. 2007; Prieto et al. 2007, 2008; Sánchez-Avila et al. 2010, 2011). Different-sized “twisters” are commercially available (10 mm × 0.5 mm, 10 mm × 1.0 mm, 20 mm × 0.5 mm, 20 mm × 1.0 mm). In the literature, both 10 mm × 0.5 mm (Popp et al., 2005; Pérez-Carrera et al., 2007; Prieto et al., 2007, 2008; Sánchez-Avila et al., 2010, 2011) and 20 × 1.0 mm (Pérez-Carrera et al., 2007; Sánchez-Avila et al., 2010) have been used.

To fine tune the extraction, the adjustment of pH, the addition of inert salts such as sodium chloride, or the addition of methanol is often studied during both SPME and SBSE. In the case of PCBs, since they have no acid–base properties, the adjustment of pH seems meaningless. The addition of sodium chloride has been studied in a few works, but contradictory results have been obtained. In the case of HS-SPME using PANI-based polymer, an increase in extraction efficiency was observed after the addition of NaCl and saturated solution was used as optimum (Wang et al., 2009b), whereas a decrease in
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extraction efficiency of high-chlorinated PCBs was observed by Derouiche and coworkers in HS-SPME using PDMS polymer (Derouiche et al., 2007). Basheer and coworkers did not add any salt during SPME with PDMS (Basheer et al., 2007). In the case of methanol, an increase of extraction efficiency has been observed after the addition of methanol since methanol decreases the adsorption of PCBs onto the walls of the glassware (Popp et al. 2005; Prieto et al. 2007).

For HS-SPME applications, it is necessary to warm up the water samples and the best extraction efficiencies were obtained at 80–85°C (Derouiche et al. 2007; Wang et al., 2009b), especially in the case of mid- and high-chlorinated PCBs (Derouiche et al. 2007). In the case of direct immersion, SMPE and SBSE room temperature was applied (Popp et al. 2005; Basheer et al. 2007; Prieto et al., 2007, 2008; Pérez-Carrera et al. 2007; Sánchez-Avila et al. 2011).

The effect of stirring can also be studied. In the case of HS-SPME using PANI-based polymer, stirring had a positive effect compared with static extraction (Wang et al. 2009b), but no improvement was observed in HS-SPME using 100 μm PDMS (Derouiche et al. 2007).

Regarding the extraction time, it is often linked to whether the mode of extraction is kinetic or equilibrium. Working under equilibrium conditions guarantees maximum extraction efficiency and the best reproducibility since small changes in extraction time have no effect. However, since equilibration time can be long in certain cases, working under nonequilibrium conditions is preferred. As a matter of fact, equilibration time for PCBs ranges from 30 to 120 min in the case of SPME (Wang et al. 2009b), although working under nonequilibrium conditions is sometimes preferred in some of the cited works (Wang et al. 2009b). Longer extraction periods are needed to reach equilibrium in the case of SBSE due to the higher amount of polymeric phase and, in this sense, equilibration time ranges from 2 to 24 h. Popp et al. (2005) described that 2 h was needed for equilibration of 10 mL water onto a 10 mm × 0.5 mm PDMS “twister” for PCBs with 1–5 chlorine atoms and 4–12 h for PCBs with 6–10 chlorine atoms. In this sense, nonequilibrium conditions are often chosen (Popp et al. 2005; Pérez-Carrera et al. 2007).

The extraction step is followed by a thermal (TD) or liquid desorption (LD) prior to the separation and detection. TD is most recommended since no organic solvent is necessary and all extracted compounds are introduced into the gas chromatographic system. In the case of SPME, a simple split/splitless inlet is enough but a thermal desorption unit (TDU) consisting of two programmable temperature vaporizers (PTVs) is necessary for SBSE. The use of TD is the most widely used option for PCBs since the analysis is mostly performed using GC (Nie et al. 2005; Popp et al. 2005; Basheer et al. 2007; Derouiche et al. 2007; Pérez-Carrera et al. 2007; Wang et al., 2009b; Sánchez-Avila et al. 2010, 2011). In the case of SPME, desorption temperatures range from 250°C to 300°C and desorption time from 3 to 5 min (Basheer et al. 2007; Derouiche et al. 2007; Wang et al. 2009b). On the contrary, longer desorption periods are necessary for SBSE (7–10 min) (Pérez-Carrera et al. 2007; Prieto et al. 2007, 2008; Sánchez-Avila et al. 2010). Since desorption time is usually long in the case of SBSE, cryofocusing is used to improve the chromatographic signal and cryofocusing temperatures between −50°C and 8°C have been used for PCBs (Popp et al. 2005; Prieto et al. 2007, 2008; Sánchez-Avila et al. 2010).

26.4.2 MEPS

MEPS is a recently developed technique introduced by Abdel-Rehim (Abdel-Rehim 2003, 2004; Abdel-Rehim et al. 2004) that can be combined in a fully automated way as an at-line sampling/injecting device to GC or LC, since the sorbent is directly integrated into the syringe as a miniaturized format of SPE (Said et al. 2008; Schurek et al. 2008; Jagerdeo and Abdel-Rehim 2009; Matysik and Matysik 2009; Morales-Cid et al. 2009). MEPS handles sample volumes as small as 10 μL.

In MEPS, approximately 2 mg of the sorbent is thermo-packed inside a syringe (100–250 μL). Different sorbent materials such as silica-based (C2, C8, and C18), carbon, polystyrene–divinylbenzene copolymer (PS-DVB), strong cation exchanger (SCX), or molecular imprinted polymers (MIPs) sorbents have been used. Thus, C2–C18 phases are suitable for lipophilic compounds and, for polar compounds such as acidic and basic analytes, polymeric phases (PS-DVB, SCX) could be more selective.

Prieto and coworkers (2010) used MEPS for the analysis of priority and emerging organic pollutants, including PCBs, by preconcentration using MEPS. 2 mg of C-18 sorbent was used for extraction and n-hexane:ethyl acetate (1:1) as elution solvent. 100 μL samples were loaded 10 times, and after the
loading step, 100 μL of air were loaded five times to dry the adsorbent. Finally, analytes were eluted in two steps, 1 × 25 μL and 1 × 50 μL. To avoid carryover, the C-18 sorbent was washed with 10 × 100 μL of the elution solvent before further analysis.

26.4.3 LLME

LLME is a simple LLE that uses only a few microliters of acceptor phase to preconcentrate the target analytes. Four main categories of LPME are found: (i) cloud-point extraction (CPE), (ii) single-drop microextraction (SDME), (iii) MLPME, and (iv) DLLME (Pena-Pereira et al. 2010; Sarafraz-Yazdi and Amiri 2010).

Owing to the nature of the extractant in CPE, aqueous solution of micelles, CPE is more suitable coupled to reverse-phase high-performance liquid chromatography (RP-LC) and no application of CPE to the extraction of PCBs has been found in the most recent literature for the past 5 years. No application of SDME to PCBs for the past 5 years has been found and, therefore, neither CPE nor SDME will be mentioned further.

26.4.4 DLLME

DLLME, first introduced by Rezaee et al. (2006), is based on the use of a ternary solvent system consisting of an aqueous phase and a mixture of two organic solvents: one that is water-miscible and acts as a dispersing agent and the other that is water-immiscible but miscible with the dispersing agent and of higher density, the extractant agent (Etxebarria et al. 2012).

The dispersant–extractant mixture is in contact with the aqueous phase within a conical tube and the formation of an emulsion, which maximizes the interface between the sample and the extractant, is readily observed. Once the extraction period is over, the mixture is centrifuged and the separation into two phases is obtained. The extracting phase containing the target analytes settles at the bottom of the conical tube and it is known as the sedimented phase.

One of the most important variables in DLLME is the extraction solvent that must have low solubility in water, low toxicity, an appropriate melting point close to or below room temperature and a density less than that of water. Moreover, it must be able to extract the target analytes (Dai et al. 2010). Dai et al. (2010) studied four different extraction solvents (n-tetradecane, n-hexadecane, 1-dodecanol, and 1-undecanol). 1-Undecanol provided the best extraction efficiency. When ECD is used, the extraction solvent must not be halogenated. In this sense, Rezai et al. (2008) evaluated carbon disulfide and chlorobenzene, with a much lower response factor than that of the PCBs in ECD. Chlorobenzene displayed higher extraction efficiency.

Another variable studied during DLLME is the volume of the extraction solvent. Dai et al. (2010) studied different volumes of 1-undecanol (5, 8, 10, 15, and 25 μL) and, although, 5 and 8 μL provided similar enrichment factors, the recovery was better using 8 μL. Similarly, Rezaei et al. (2008) studied the effect of the extraction solvent (chlorobenzene) in the 10–30 mL range. When the volume of the extraction solvent was high, a decrease of the enrichment factor was observed and 10 mL was chosen as optimum. This volume could not be set lower than 10.0 μL, on the grounds that the sedimented phase volume would become less than 5.0 μL, causing difficulties in its removal with a microsyringe and encountering systematic errors.

The dispersive solvent must be miscible with both the extraction solvent and the aqueous sample. Dai et al. (2010) evaluated four different dispersive solvents (acetonitrile, acetone, methanol, and isopropanol) and acetonitrile provided the best enrichment factors. In the work by Rezaei et al. (2008), methanol, acetonitrile, and acetone were studied; similar enrichment factors were obtained and, finally, acetone was chosen for its lower toxicity and cost. In the case of the volume of the dispersive solvent, the volume must be high enough for the cloudy state to be formed but without altering the solubility of target analytes in the water solution (Dai et al. 2010).

One way to avoid the use of the dispersive solvent is to promote the analyte phase transfer by means of ultrasounds (ultrasound-assisted emulsification–microextraction (USAEME)) (Dasgupta et al. 2011) or mechanically (vortex-assisted liquid–liquid microextraction (VALLME)) (Ozcan 2011).

Different works have studied the effect of the addition of NaCl. Dai et al. (2010) observed an increase of the enrichment factor up to 2% of NaCl, but larger amounts of NaCl did not improve further the
enrichment factor (Dai et al. 2010), whereas Rezaei et al. (2008) did not observe any improvement of the enrichment factor after the addition of NaCl in the 0–5% range. Finally, Ozcan et al. (2009) observed a decrease in extraction efficiency after the addition of NaCl.

In the case of the extraction time, it has been observed that the transport of analytes from the aqueous phase to the extraction solvent was so fast that equilibrium can be achieved in a short period of time (Dai et al. 2010) and extraction times are very short, lower than 1 min in some cases.

DLLME finishes with the centrifugation and the recovery of the organic droplet containing the target analytes. One way to recover the organic solvent containing the target analytes is the solidification of the extractant, named DLMME, based on the solidification of the floating organic droplet or DLMME-SFO. After the extraction and centrifugation, the test tube where extraction takes places is placed into an ice bath and the solidified floating organic solvent is recovered with a small medical nipper (Dai et al. 2010).

### 26.4.5 MLPME

MLPME was introduced years ago as a simple and inexpensive alternative to traditional LLE methodology and its development is still ongoing. The extraction takes place between the aqueous sample (donor phase) and a microvolume of acceptor phase, protected by a membrane that avoids the mixture of the two phases and acts as a selective barrier between the phases (Psillakis and Kalogerakis 2003). The main advantages over conventional LLE are the avoidance of emulsion formation, the lack of the phase separation step, and the use of modules with a high surface-area-to-volume ratio (Moreno Cordero et al. 2000).

There are two main categories depending on the nature of the membrane: porous or nonporous membrane techniques.

#### 26.4.5.1 Porous Membranes

When using porous membranes, two techniques can be distinguished: (i) supported liquid membrane (SLM) extraction and (ii) microporous membrane liquid–liquid extraction (MMLLE). While SLM is a three-phase extraction system with an organic phase immobilized in a porous hydrophobic membrane separating two aqueous phases (Zorita et al. 2007), MMLLE is a two-phase membrane extraction technique with one aqueous sample and an organic acceptor phase inside the microporous membrane, where the same organic liquid is immobilized in the membrane pores. In this sense, SLM is mainly used for the analysis of acidic or basic polar compounds that are easily protonated and often have low log $K_{ow}$ (Kou et al. 2004; Yamini et al. 2006), whereas MMLLE best suits the extraction of neutral and/or more hydrophobic organic compounds with high partition coefficients to the organic phase (Jönsson and Mathiasson 2000; Fontanals et al., 2006), such as PCBs.

The most commonly used porous hydrophobic membranes are polypropylene (PP), polytetrafluoroethylene (PTFE), and polyvinylidene difluoride (PVDF) (Jönsson and Mathiasson 2000). PP membranes are much highly desirable as they provide unique suitability and performance for analyte extraction, in terms of high porosity that can enhance mass transfer, compatibility, and stability when used with wide range of organic solvents (Barri and Jönsson 2008). Besides, among the several configurations applicable (flat sheet, spiral wound, and hollow fiber; either rod-like or U-shaped) (Jönsson and Mathiasson 2001), hollow fiber-based SLM, also termed as hollow fiber liquid-phase microextraction (HF-LPME), is the most popular and stable (He et al. 2004). HF-LPME can be performed in both the static and dynamic modes. In the static mode, the HF is supported by a guiding tube into the sample, while in the dynamic mode, a conventional microsyringe with the HF attached to its needle is connected to a syringe pump to perform the extraction (Basheer and Lee 2004).

Li et al. (2008) used dynamic HF-LPME for the extraction of PCBs in river water. 3 mL of toluene and 3 mL of ultrapure water were withdrawn into a 10 mL microsyringe. The syringe needle was inserted into the hollow fiber segment and the assembly was then immersed into the organic solvent. After impregnation for 5 s, the 3 mL water was discharged from the syringe to displace the organic solvent inside the hollow fiber. The needle was immediately immersed into the stirred aqueous sample (10 mL), and the syringe was secured. Then, the plunger was depressed or retracted at a constant speed to inject or withdraw the organic solvent. The above extraction process was repeated within 15 min.
Another modification is polymer-coated hollow fiber microextraction (PC-HFM). Basheer et al. (2007) developed a porous polypropylene hollow fiber membrane coated with a conjugated polymer used as an on-site sampling device for the extraction of PCBs. The derived polymers are the copolymers consisting of phenylene with the long alkyl substitution \((R=\text{C}_{12}\text{H}_{25})\) and \(\text{OCH}_2\text{C}_6\text{H}_5\) groups on opposite sides (para) of the benzene ring. The three different conjugated polymer coatings synthesized provided better extraction efficiency than commercially available PDMS. The best extraction time was set in 10 min and the best extraction efficiency was obtained under stirring compared to the static mode. The influence of the sample pH on extraction efficiency was investigated and lower amount of the analytes were extracted at pH 2 and 12 and most of the PCBs exhibited better adsorption at pH 8 (real seawater pH). Addition of salt did not have any influence in the extraction efficiencies. The best desorption solvent was \(n\)-hexane and 20 min sonication was sufficient for quantitative desorption of the target analytes.

### 26.4.5.2 Nonporous Membranes

The main difference between MASE and microporous membrane extraction techniques is that the membrane used in MASE is a low-density polyethylene (LDPE), dense PP, PDMS silicone rubbers, and asymmetric composite polymeric membranes that are composed of a thin layer of silicone and another layer of polycarbonate (PC) or a relatively thick support layer of porous PP.

MASE was applied to the determination of PCBs together with other organic pollutants by Prieto et al. (2008). High-density polypropylene membrane bags (4 cm long with a wall thickness of 0.03 mm and an internal diameter of 6 mm) were used through all the experimentation and seven different solvents \((n\)-hexane, cyclohexane, \(n\)-heptane, ethyl acetate, toluene, acetone, and dichloromethane) were studied. Cyclohexane provided the best results for PCBs but since a simultaneous extraction of PCBs together with PAHs, polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), phthalate esters (PEs), and nonylphenols (NPs) was carried out, cyclohexancethyl acetate (1:1, v/v) was finally chosen for consensus. Other variables studied were the addition of methanol and NaCl, extraction temperature, stirring speed, and extraction time. After an experimental design optimization approach, NaCl and MeOH were fitted at 20%, stirring at 500 rpm, and extraction temperature at 50°C. According to the time profile, equilibrium was reached after 20–40 min for all the PCBs, but 60 min extraction was finally chosen for the simultaneous extraction of all the families studied under equilibrium conditions.

### 26.5 Separation and Detection

Though the analytical performance is typically focused on the type of detection method employed, this only represents the last step of the analytical method. In the case of the analysis of PCBs, as mentioned in the introduction, there are specific requirements demanding very sensitive methods and especially for the determination of the dioxin-like congeners. Broadly speaking, the two key points are the resolution of the chromatographic system and that of the mass spectrometer. However, a considerable body of literature exists describing a wide range of methods and techniques for the determination of PCBs in various matrices, including the use of high-performance liquid chromatography (HPLC) (Pietrograndi et al. 2000), thin layer chromatography (TLC) (Gruenwedd and Whitakea 1987), high-performance liquid chromatography gas chromatography/mass spectrometry (HPLC–GC/MS) (Liu et al. 2000), fluorometry (Fernandez et al. 1998), immunoassays, IAs, (Ahmed 2003), biosensors (Mascini 2001), supercritical chromatography (SFC) (Font et al. 1996), electrokinetic chromatography using cyclodextrin (Garcia-Ruiz et al. 2001), and gas chromatography–flame ionization detector (GC–FID) (Majid and Sparks 2002).

Regarding the chromatographic system, capillary GC column has made it possible to achieve lower detection limits and better separation of individual PCB congeners for quantification (see Table 26.1) (Garcia-Flor et al. 2009; Półkowska et al. 2011), although complete separation of all congeners on a single column has not yet been achieved. Selectivity is a necessary condition for obtaining reliable measurement results. In the case of chromatographic analysis—the “classical” technique for determining PCBs—selectivity is represented by the separation factor, the value whose values is a function of the
difference in retention times and chromatographic peak widths. The parameters affecting chromatographic selectivity are

- The type of column used
- The chromatographic separation parameters (e.g., temperature program, gas flow rate)

With a suitable chromatographic column, the analyte signal can be distinguished from the interferent signal. If an inappropriate column is used, the materials to be assayed may well be determined in combination. In the case of PCBs, these are often determined as the sum of CB 28 + CB 31 or CB 138 + CB 162; moreover, the order of elution of the isomers CB 28 and CB 31, CB 138, and CB 153 is frequently confused. The separation of CB 31 from CB 28 cannot be done on a column containing stationary phase DB-17, but is possible with a DB-1701 column. The commonly used capillary columns (DB-5, DB-1701, SE-54, SIL-8, SP-2330, and CP-SIL-9) provide poor or no resolution for the following sets of congeners: 15/18, 28/31, 49/52, 77/110, 84/90/101, 118/149, 138/163/164, 105/132/153, 170/190, and 182/187 (García-Flor et al. 2009; Oliveira et al. 2011).

Nevertheless, the trend is toward congener-specific analysis by capillary GC. Recent advances include analytical methods that are able to quantify individual PCB congeners. EPA method 1668 (US EPA Method 1668) is the current methodology used to determine individual PCB congeners in water, soil, sediment, and tissues by GC and HRMS. Revision A of Method 1668 has been expanded to include congener-specific determination of more than 150 chlorinated biphenyl (CB) congeners (Method 1668A) (US EPA Method 1668A). The 12 PCBs designated as toxic by the World Health Organization (WHO) (also known as dioxin-like PCBs) and the earliest and latest eluted congener at each level of chlorination (LOC CBs) are determined by the isotope dilution quantification technique, while the remaining congeners are determined by the internal standard quantification technique. Immediately prior to injection, labeled injection internal standards are added to each extract and an aliquot of the extract is injected into the gas chromatograph. The analytes are separated by GC and detected by a high-resolution (>10,000) mass spectrometer. Two exact mass-to–charge ratios (m/z) are monitored at each level of chlorination throughout a predetermined retention time window. Another method for PCB analysis is EPA Method 508A (US EPA Method 508A) of the US EPA, which converts all the PCBs to decachlorobiphenyl (DCP). Method 508A is a screening method for quantifying total PCBs. The method is likely to show interference because of perchlorination of biphenyl or related compounds and because the method cannot quantify either the individual commercial Aroclors in a PCB mixture or the individual congeners present.

Regarding the mass spectrometers, HRMS with electron impact ionization (EI) is the method of choice for PCB determination because it is a technique capable of providing the required selectivity and sensitivity. GC–HRMS is currently the reference technique for the analysis of these compounds providing limits of detection on the low femtogram level by operating in selected ion monitoring (SIM) mode. In addition, high selectivity is obtained as a result of the high resolution applied. Ruiz-Fernández et al. (2012) analyzed 36 chromatographic peaks, with reference to 49 PCB congeners (25 as single congeners) among others in environmental samples: 11 trichlorobiphenyls (CBs 18, 17, 16 + 32, 26, 25, 28 + 31, 20 + 33, 22), 17 tetrachlorobiphenyls (CBs 52, 49, 47 + 48, 44, 42 + 59, 41 + 64 + 71, 40, 74, 70, 66, 56 + 60, 77), 14 pentachlorobiphenyls (CBs 93 + 95, 91, 84 + 90 + 101, 99, 97, 87 + 115, 85, 110, 118, 105), six hexachlorobiphenyls (CBs 136, 151, 149, 153, 138 + 164), and one heptachlorobiphenyl (CB 176). Six 13C-labeled PCBs were added to the samples as internal standards before extraction: 13C-CB28 was used for the quantification of mono-CB, di-CB-, and tri-CB congeners, 13C-CB52 for the tetra-CBs, 13C-CB101 for the 5-CBs, 13C-CB153 for the 6-CBs, and 13C-CB180 for 7- and 8-CBs. GC–MS detection limits were 1 pg for all congeners, whereas at 0.6 pg level, 80% of them were detectable. Isotopic dilution is widely used for quantification purpose with HRGC–HRMS (Roach et al. 2009; Jotaki et al. 2011; Oliveira et al. 2011) or with other techniques as GC–MS (Moret et al. 2005; Manodori et al. 2006; Gasperi et al. 2009) or GC–MS/GC–ECD (Berrojalbiz et al. 2011). Isotopic dilution can be treated as a specific variant of the internal standard technique. Its specificity depends upon the fact that, before analysis, a known mass of a standard chemical compound (or mixture of standard compounds) is added to the sample; standards have the same physicochemical properties as the analytes but their molecular
masses differ from those of the analytes in their isotope content. MS enables simultaneous estimation of the content of both analytes in a sample and their isotope-labeled analogs added to the sample during its preparation for analysis. The use of mass spectrometer (MS) makes it easy to identify the individual peaks on the basis of a selected ion. The labeled compound typically elutes 1–3 s prior to its unlabeled analog. This allows for better analyte identification in complex samples, where retention times may be shifted due to coextractable compounds in the sample or interferences that may be present in the chromatograms. The ability to measure the peak-area ratios of native versus labeled compounds in the sample increases precision and accuracy. These same analysis methods (HRGC–HRMS with isotope dilution) can also be found in five different media simultaneously: dissolved, suspended sediment, bed sediment, catfish, and blue crab (Howell et al. 2011) or air, lake water, tributary river water, treated effluents from the largest WWTP (wastewater treatment plant), and sediment (Bogdal et al. 2010) or sewage sludge (GUO et al. 2009). In the special case of water analysis, there are also many examples of the use of HRGC–HRMS. Roach and coworkers determined 12 dioxin-like PCBs in seawater column samples from Sydney harbor (Roach et al. 2009) and in the study by Oliveira and coworkers, 209 PCBs were analyzed from five tributaries of U.S. Lake Ontario with 21 pg/L of method detection limit (MDL) (see Table 26.1) (Oliveira et al. 2011). In the same way, Joseffson and coworkers analyzed 22 PCBs in river waters from Sweden with low detection limits (Joseffson et al. 2011).

Nevertheless, HRMS has a high operating cost and requires skilful staff. To reduce or at least alleviate the costs of the analysis, alternative methods have been investigated. The most used technique for PCB determination is GC coupled with low-resolution spectrometer (GC–MS) (Cocco et al. 2011; Djedjibegovic et al. 2010; Gasperi et al. 2009; Grimalt et al., 2009; Manodori et al. 2006; Maruya et al., 2009; Minomo et al. 2011; Popp et al. 2005; Zhang et al. 2011; Zorita et al. 2006). Single MS is a very selective technique that is widely employed for pollutant analysis but its sensitivity cannot be compared with that of HRMS instruments. Although there are some ways to enhance sensitivity optimizing the SIM program using the autoSIM option (diminishing the number of ions displayed in each window and therefore, increasing in sensitivity) (Lacorte et al. 2009), the two common ways for increasing the sensitivity of chromatographic determinations are increasing of the sample size used for the analysis, and/or injecting large volumes of sample extracts into the GC column. Large-volume injection with a PTV appears to be a good alternative. The PTV inlet collects each portion of an injected sample inside a 120 μL liner until the entire quantity of sample is obtained. During the hold, the sample is held at a programmable temperature as low as −60°C using CO₂ cooling, or as low as −160°C using liquid nitrogen. When the complete sample is collected, the PTV heats and delivers the sample onto the column. The PTV can be used with either a septum or septumless head, operating in split, splitless (Ozcan et al. 2009, 2011; Norli et al. 2011), or solvent vent modes (Schellin and Popp 2003; Turrio-Baldassarri et al. 2005; Prieto et al. 2010). In the solvent vent mode, analytes are thermally trapped in the liner while the solvent is removed. With the solvent gone, the liner volume can be used for another injection. Injection can be repeated several times to concentrate the analytes from a large sample volume. After injection and solvent removal, the analytes are transferred to the column. This can replace the need for offline preconcentration and minimize the loss of sample (Jotaki et al. 2011). In the case of Prieto and coworkers (2010), two sample preparation methods based on MEPS and SPE combined by LVI–GC–MS run have been developed. Both methods permit the accurate multiresidue determination of 41 organic pollutants (14 PCBs) in water at low levels (ng/L) for a 100 mL and 800 μL (SPE and MEPS sample volumes) without the need for standard addition procedure (see Table 26.1). Ozcan and coworkers has outlined the successful development and application of a novel extraction procedure for the determination of PCBs in water samples by using GC–MS with a 200 μL PTV injection (Ozcan et al. 2009).

Some researchers using this technique coupling with retention time locking (RTL) tool with forensic purpose to screen water samples covering more than 500 organic microcontaminants (Etxebarria et al. 2009). It is worth noting that this tool provides remarkable screening performance; it allows the easy and precise identification of each compound from GC current data by reporting all matches or probable hits. For instance, Almeida and coworkers (Almeida et al. 2007) and Quiroz and coworkers (Quiroz et al. 2009) use GC–MS system with large-volume injections or thermal desorption, respectively, for environmental forensic analysis. In both cases, capillary gas chromatography coupled to mass spectrometry combined with retention time locking libraries (GC–MS–RTL), was shown to be a fast, reproducible,
and robust methodology for screening hundreds of nontarget semivolatile compounds from a large number of unknown environmental matrices simultaneously. The results obtained from the application of the proposed methodology to the screening of water, sediment matrices, snow and coastal waters for organic microcontaminants demonstrated the remarkable selectivity and sensitivity of the methodology at the ultra-trace level.

ECD coupled to a GC is still one of the most affordable techniques for the analysis of PCBs. There are a lot of works where PCBs are determined in waters and other matrices by means of ECD (Rezaei et al. 2008; Dai et al. 2010; Gschwend et al. 2011; Liu et al. 2011), although the unequivocal identification of the target compounds is assured if the most selective MS detector is employed (Carvalho et al. 2009; García-Flor et al. 2009; Berrojalbiz et al. 2011). As a matter of fact, the main disadvantage of the EDC is that the identification of analytes relies solely on their retention time ($t_R$). As a result, any compound interfering or coeluting with the target analytes can prevent accurate quantification of those analytes.

Other researchers (Wang et al. 2009b; Jiang et al. 2011) use a microcell ECD (μECD). Basically, it is the same ECD but the detection zone volume, 10 times smaller than any other ECD, assures detection with extremely low concentrations. Moreover, higher linear velocities through the detection zone reduce analyte residence time, decrease the chance of cell contamination, and improve uptime. This fact, together with a variable sampling rate from 5 to 50 Hz, makes μECD ideal for fast chromatography (Palmer et al. 2011). For example, 209 PCB congeners were determined in sample waters using GC–μECD. The MDL was about 32.3 ng/L. An extra chromatographic column was used to confirm the analysis.

Probably among alternative methods to HRGC–HRMS, tandem mass spectrometry (MS/MS) using ion-trap mass analyzers (IT–MS/MS) is one of the most promising techniques (Li et al. 2005; Herbert et al. 2006; Ding et al. 2008). The limited resolution of the ion-trap is compensated by the possibility of operating in the tandem mode. In addition to this, low enough limits of detection are achievable due to the significant increase of the signal-to-noise ratio provided by this operating mode. Although the sensitivity achieved by ion-trap MS–MS is lower than that provided by HRMS, good values have been reported (Eljarrat et al., 2008; Hong et al., 2009). Besides, sensitivity can be improved by increasing the dicing gas pressure inside the trap that produces an enhancement of the efficiency of both precursor ion-trapping and fragmentation. Nowadays, GC–MS systems with two different ion-trap configurations, internal and external ionization, are commercially available. Both have been indistinctly used for the determination of dioxin-like PCBs in environmental samples, such as sewage effluents (Cunnliffe and Williams 2006) or wastewater (Kemmochi 2003) providing low limits of detection. Finally, the use of a PTV coupled to GC–IT–MS/MS has been reported to achieve better analytical performance (Fuoco et al. 2009).

All the characteristics mentioned above have made IT–MS/MS a very competitive and widely used technique. Comparison study between GC–ECD and GC–IT–MS/MS demonstrated that ECD provides less confidence of identification than mass spectrometry (Verenitch et al. 2007). As a result, coeluting peaks or interferences becomes an issue affecting ECD's data quality. For example, CB congeners 28 and 31, both trichlorinated biphenyls, coelute, and with identical mass spectra, they had to be quantified as a sum using single mass spectrometry, but quantification was possible using tandem mass spectrometry. Similarly, coeluting CBs 70 (tetraCB) and 95 (pentaCB) can be accurately quantified by IT–MS/MS technique due to the differences in their precursor ions.

In terms of the limit of detection, Verenitch et al. (2007) found that HRMS provided approximately 10 times lower values. However, the specificity of tandem mass spectrometry provided comparable data in terms of accuracy and precision.

In the last decade, comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC × GC–MS) has been widely applied to the analysis of organic pollutants in complex samples. The key features of GC × GC–MS are large separation power, improved detectability due to the signal enhancement after zone compression, group-type separation using both GC × GC separation and mass chromatography, and identification capability with mass spectral information (de Vos et al. 2011; D’Archivio et al. 2011). The high-speed time of flight mass spectrometer (TOF-MS) has been suggested as the best candidate MS detector for GC × GC systems because it is one of the detectors with the highest acquisition rates. Several researchers have reported the applicability of moderate acquisition rate instruments, such as quadrupole MS (QMS) (e.g., 20 Hz) as the following candidate MS for GC × GC, even with the limited mass range and lower acquisition rate.
Recently, GC × GC coupled with a high-resolution TOF-MS (HRTOF-MS) that allowed accurate mass measurement (mass measurement with uncertainties of a few mDa) was applied to the analysis of organic pollutants in environmental samples (Hoh et al. 2007; Dasgupta et al. 2011). Although the maximum acquisition rate of the HRTOF-MS is 20–25 Hz, GC × GC–HRTOF-MS can provide additional important features such as (a) accurate mass measurement for full mass range (e.g., m/z 45–500), (b) higher selectivity and sensitivity using a very narrow mass window (0.02–0.05 Da), and (c) calculation of elemental composition from accurate mass molecular ion, resulting in a reliable identification. Increased selectivity with the GC × GC–HRTOF-MS allows a group-type separation of a selected chemical class, for example, PCBs, polychlorinated naphthalene (PCNs), and PCDFs.

GC × GC–TOF-MS fulfills the tight requirements of the analysis of complex samples at the levels needed for priority pollutant determination in a single analysis as the EPA Method 1613 (US EPA Method 1613) for the quantification of the 17 PCDDs and PCDFs (Hoh et al. 2007). Additionally, the GC × GC–TOF-MS provides a quick and convenient screen for numerous pollutant classes, such as PCBs, which may be present in samples arising from toxic waste disposal, as it has been done to determine the efficiency of the incineration and safety (de Vos et al. 2011).

The combination of already-mentioned new injection devices such as thermal desorption has opened new possibilities to the use of sorptive extraction methods. A TD–GC × GC–HRTOF-MS method for the determination of POPs at pg/L to ng/L levels in river water was described by Ochia et al. (2011). The method provides many practical advantages such as a small sample volume (50 mL), simplicity of the extraction, and higher sample throughput with parallel extraction (typically 20 samples). Also, it is solvent-free and highly selective and sensitive (LOD: 10–44 pg/L). The method was successfully applied to the determination of 16 OCPs in a river water sample. Moreover, the method allows screening of nontarget compounds using accurate mass measurement in GC × GC. Twenty nontarget compounds, for example, pesticides, PAHs, PCBs, and PPCPs, were positively or tentatively identified in the same sample. Although most applications of TOF/MS are coupled to GC × GC, a few applications of one-dimensional GC coupled to this detector can also be found in the literature (Park et al. 2009; Portoles et al. 2011).

Another application of GC × GC is the determination of chiral PCBs (Bucheli and Brändli 2006). Out of the 209 PCB congeners, 19 tri- and tetra-ortho congeners exist as atropisomers (i.e., stereoisomers from hindered rotation about a single bond) at room temperature; these chiral PCB congeners are released into the environment as racemic mixtures. Confident determination of nonracemic chiral PCBs distribution in animal tissues, waters, and food extracts could provide crucial information relevant to biotransformations and selective bioaccumulation. Recently, it was shown that with rigorous sample preparation and multiple injections, 1P-GC equipped with cyclodextrin columns can partially resolve 15 out of 19 atropisomeric PCBs (Frame 1997). However, in real-life samples, this cannot be achieved because 1P-GC cannot separate the atropisomeric PCBs from both regular PCB congeners and from the remaining matrix components. This leads to poor resolution and poor identification of enantiomeric chiral PCBs. In one of the early experiments, a narrow-bore β-cyclodextrin primary column and a liquid crystal secondary column to separate atropisomeric PCBs was used. Although not all atropoisomers were resolved in real samples, the GC × GC method was capable of separating nine chiral PCBs coeluters in 1P-GC. More recently, Bordajandi et al. (2005) developed a GC × GC method for the simultaneous analysis of chiral PCBs and toxicity indicator PCBs. The group was successful in separating and identifying nine atropisomeric PCBs and all 12 nonortho and seven EU toxicity marker PCB congeners from food samples (salmon and dairy products). When compared to conventional 1P-GC analyses, GC × GC decreased analysis time (due to minimized sample preparation procedures) and provided the higher-resolution chromatograms required when analyzing complex food samples. Presently, no single column can separate all 209 PCB congeners in one run. The most optimal HRGC–MS system in an intercomparison study utilizing 27 different high-resolution GC (HRGC) systems including 20 different columns, exhibited coelutions of 34 PCBs (Harju et al. 2003). Although this is a significant achievement in 1P-GC, it is unsatisfactory in routine analysis where certain target congeners are included in the the coeluted groups of PCBs. In addition, the number of observed coelutions would certainly rise when analyzing PCBs in natural samples.
Another typical detector coupled to GC × GC is µECD. Harju et al. used GC × GC–µECD to separate 194 PCB congeners in 240 min (Harju et al. 2003). Unfortunately, the separation generated very large data files (up to 500 MB each) and at times took up to 2 h to process. The most recent version of the commercially available GC × GC software minimizes this limitation by significantly shortening data processing times.

### 26.5.1 Other Methods

As mentioned previously, the reference method to analyze PCBs in environmental samples (HRGC–HRMS) might be less useful when rapid data on PCBs from a large set of test samples is required. Immunoassay and bioassay methods can reduce the costs by 50% or more and have been used to analyze critical samples and samples collected from hazardous sites where very fast analysis is needed.

The main advantages of biological approaches are rapid analysis, sample pretreatment is simple or not required, possibility of in situ analysis, low volumes of sample required, and use of solvents low or not required. The main disadvantages of these types of methods are the inability to use labeled internal standards to correct for recovery and the determination of congener profiles for source apportionment. Spiked samples, similar to standard addition quantitative analysis, permit recoveries to be estimated, overcoming this problem. Briefly, in the immunochemical techniques, binding properties of an antibody (Ab) to an antigen (Ag) have been used for the development of a wide variety of analytical techniques applicable in rapid environmental control for the measurement of both single and multiple analytes. Immunoassays offer a number of advantages in green environmental analysis over conventional methods because they can provide fast, simple, and cost-effective detection, with sensitivity in most cases comparable to conventional techniques and requiring no or minimal quantities of solvent, with minimal sample pretreatment required (so reducing waste) and reduced energy consumption during sample analysis. In addition, more advanced formats (e.g., immunoassays) can be designed to operate online in the field. The main limitations encountered with these techniques are sometimes poor thermal and chemical stability of immune reagents, cross-reactivity between structurally related compounds, and possible matrix effects. Current development is therefore focused on the application of new materials to improve the stability and the specificity of immune reagents. At present, most immunochemical advances in environmental analysis have been carried out using enzyme-based immunoassays (EIAs), especially in immune sensing.

A biosensor is defined by IUPAC as a self-contained integrated device that is capable of providing specific quantitative or semiquantitative analytical information using a biological-recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element. Biosensors offer capabilities for rapid, miniaturized, online, and at-site analysis, with minimal waste production and energy cost. Several reviews on biosensors and biological techniques for environmental analysis have been published in recent years (Kurosawa et al. 2006; Borisov and Wolfbeis 2008).

The development of a rapid and inexpensive screening method for PCBs remains a high priority. A number of studies have reported the utility of reporter gene assay systems as possible alternatives for screening PCBs in human blood plasma (van Wouwe et al. 2004) and river sediments (Vondracek et al. 2001). Recently, a new sensitive and rapid reporter gene assay (DR-cell assay) using a genetically engineered stable cell line designated DR-EcoScreen cells was developed (Takeuchi et al. 2008). The minimal MDL with little variance data (within CV 10) suggests that the DR-cell assay might be more sensitive to PCBs than other reporter gene assays (Kojima et al. 2011).

### 26.6 Concluding Remarks

As mentioned in the introduction, the analysis of PCBs is still a challenging matter in analytical chemistry. On the one hand, many new procedures focused in the ease of lab work and to assure higher sensitivities and better recoveries have been found. On the other hand, the dilemma is played in the ground of the type of equipment required to perform the analysis: the big and expensive instrumentation and the instrumentation usually found in average laboratories.
Since the requirements for the analysis of PCBs can be slightly different, there is enough space for many of the options described above. Making use of the precise quality assurance/quality control system, many laboratories can provide good and useful analytical results with, for instance, the combination of a SBSE and a TD–GC–MS, which can be considered an affordable investment. However, for some tighter requirements, more elaborate instrumentation such as GC–HRTOF should be used and this will be only feasible for a selected type of labs.

REFERENCES


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